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Distinct Types of T-cell Help for Induction of a Humoral Immune Response to *Streptococcus pneumoniae*-Clifford M. Snapper

Studies have indicated that purified, soluble polysaccharide antigens can elicit T cell-independent Ig responses *in vivo*, although these responses can be modulated by T cells in a non-cognate manner. Relatively little is known, however, concerning the parameters that regulate polysaccharide-specific, as well as protein-specific, Ig isotype responses to an intact, extracellular bacterium. To address this issue, we utilized a heat-killed intact *Streptococcus pneumoniae* to determine the parameters that regulate Ig isotype responses specific for the bacterial cell wall protein, PspA and for the phosphorylcholine (PC) determinant of the cell wall teichoic acid (C-polysaccharide). We demonstrate that in contrast to the anti-PspA response, the anti-PC response develops with more rapid kinetics and does not demonstrate immunologic memory, properties consistent with purified polysaccharide antigens. Nevertheless, both the IgG anti-PspA and IgG anti-PC responses are dependent upon endogenous TCR-a/b3+3, but not TCR-g/d3+3, T cells. The anti-PspA response is strictly dependent upon CD43+3 T cells, whereas helper activity for the anti-PC response is mediated by both CD43+3 and CD83+3 T cells. To further explore the nature of the T cell help for these two responses we utilized H-Y transgenic mice that had been crossed with TCR-a3-/-3 mice (H-Y-a3-/-3). These mice expressed no endogenous TCR, possessed relatively normal numbers of CD43+3 and CD83+3 T cells, and had normal numbers of B cells. When H-Y-a3-/-3 mice were immunized with R36A, no detectable anti-PspA response was observed3 3consistent with the absence of MHC-restricted, antigen-specific T-cell help predicted for this mouse model. The absence of an antibody response to PspA was associated with a complete absence in germinal center formation. By contrast, the anti-PC response to R36A in H-Y-a3-/-3 mice was similar to controls. When H-Y-a3-/-3 mice were acutely depleted of T-cells or injected with CTLA4Ig before R36A immunization, a significant drop in R36A-induced serum anti-PC titers was observed, comparable to that seen similarly-treated wild-type mice. Thus, T cells appear to augment the anti-PC response in a TCR-non-specific manner, in the absence of germinal center formation, consistent with the lack of PC-specific memory. To further investigate the nature of the T cell help for the anti-PspA and anti-PC responses, we generated a relatively pure population of immature, CD11c3+3, CD83-3 (myeloid) DCs by culturing DC-enriched bone marrow (BM) cells in granulocyte-macrophage colony stimulating factor (GM-CSF). DCs were incubated *in vitro* with type 14 *S. pneumoniae*, washed thoroughly to remove free bacteria, and injected i.v. into naïve mice. *S. pneumoniae*-pulsed DCs elicited a primary anti-PspA response as well as PspA-specific memory. In addition, pulsed DCs elicited both an anti-PC and anti-Pn14 response. All three responses required viable DCs. Importantly, both the primary anti-PspA and anti-PC responses elicited by pulsed DCs were T cell-dependent. DCs from MHC class II3-/- 3mice, which were pulsed with *S. pneumoniae* and transferred into naïve mice, were markedly defective at eliciting a primary anti-PspA response, as well as PspA-specific memory. By contrast, DCs lacking MHC class II elicited an anti-PC response comparable to that observed using wild-type DCs. In a complementary study, pulsed DCs transferred into an allogeneic recipient failed to elicit an anti-PspA response but induced an anti-PC response comparable to that observed when the same DCs were transferred into a syngeneic host. These data are thus consistent with the notion that the anti-PspA response requires cognate, MHC class II-restricted interactions between DCs and T cells, whereas the anti-PC response involves non-cognate activation of TCR-non-specific T cells.